

AMENDMENTS TO THE CLAIMS

1. **(Currently amended)** An isolated recombinant human arginase I, comprising ~~having~~ substantially the same amino acid sequence as set forth in ~~Fig. 2C~~ SEQ ID NO: 9 and having a purity of 80-100%.

2. **(Currently amended)** The recombinant human arginase I according to claim 1 further ~~having~~ comprising six ~~additional~~ histidines attached to the amino terminal end thereof.

3. **(Currently amended)** The recombinant human arginase I according to claim 1 ~~or 2~~ having a specific activity of at least 250 I.U./mg.

4. **(Original)** The recombinant human arginase I according to claim 3 having a specific activity of 500 to 600 I.U./mg.

5. **(Currently amended)** The recombinant human arginase I according to claim 4, comprising a modification resulting that results in an *in vitro* plasma half-life of at least approximately 3 days.

6. **(Currently amended)** An isolated recombinant human arginase I according to claim 1, ~~or 2~~ having a purity of at least 90%.

7. **(Original)** The recombinant human arginase I according to claim 5, wherein said modification is pegylation.

8. **(Original)** The recombinant human arginase I according to claim 7, wherein said pegylation results from covalently attaching at least one polyethylene glycol (PEG) moiety to said arginase using a coupling agent.

9. **(Original)** The recombinant human arginase I according to claim 8, wherein said coupling agent is selected from the group consisting of 2,4,6-trichloro-s-triazine (cyanuric chloride, CC) and succinimide propionic acid (SPA).

10. **(Currently amended)** A method of producing recombinant protein comprising:

- (a) cloning a gene encoding said protein;
- (b) constructing a recombinant *Bacillus* ~~*subtilis*~~ *subtilis* strain for expression of said protein;

Appl. No. : Unknown
Filed : Herewith

- (c) fermenting said recombinant *Bacillus subtilis* cells using fed-batch fermentation;
 - (d) heat-shocking said recombinant *Bacillus subtilis* cells to stimulate expression of said recombinant protein; and
 - (e) purifying said recombinant protein from the product of said fermentation.
11. **(Original)** The method according to claim 10 wherein said *Bacillus subtilis* is a prophage.
12. **(Currently amended)** The method according to claim 10 ~~or 11~~ wherein said protein is human arginase I.
13. **(Currently amended)** The method according to claim 12 wherein said human arginase I ~~has~~ comprises six histidines linked to the amino-terminus thereof, and said purifying step comprises affinity chromatography in a chelating column.
14. **(Original)** The method according to claim 12 wherein said fermenting step is performed using a feeding medium consisting essentially of 180-320 g/L glucose, 2-4 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 45-80 g/L tryptone, 7-12 g/L K_2HPO_4 and 3-6 g/L KH_2PO_4 .
15. **(Original)** A pharmaceutical composition comprising an isolated and substantially purified arginase.
16. **(Original)** The pharmaceutical composition according to claim 15 wherein said recombinant human arginase is human arginase I.
17. **(Currently amended)** The pharmaceutical composition according to claim 15 wherein said recombinant human arginase is human arginase I, further comprising ~~containing~~ six additional histidines attached to the amino terminal end thereof.
18. **(Original)** The pharmaceutical composition according to claim 15, wherein said composition is further formulated in a pharmaceutically acceptable carrier.
19. **(Currently amended)** The pharmaceutical composition according to claim 15, wherein ~~said the~~ formulation of said pharmaceutical composition is in a form suitable for oral use, for a sterile injectable solution or a sterile injectable suspension.
20. **(Original)** The pharmaceutical composition according to claim 16, wherein said recombinant human arginase I has a specific enzyme activity of at least 250 I.U./mg.

Appl. No. : Unknown
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21. **(Original)** The pharmaceutical composition according to claim 20, wherein said recombinant human arginase I has a specific enzyme activity of 500 to 600 I.U./mg.

~~24.22.~~ **(Currently amended)** The pharmaceutical composition according to claim 16, wherein said recombinant human arginase I has a half-life in ~~said~~ patient plasma of at least 3 days.

~~22.23.~~ **(Currently amended)** The pharmaceutical composition according to claim ~~24~~ 22, wherein said recombinant human arginase I has a half-life in ~~said~~ patient plasma of approximately at least 1 day.

~~23.24.~~ **(Currently amended)** ~~The use of~~ A method of treatment of human malignancies, comprising administering human arginase I of claim 1 for the preparation of a medicament.

~~24.25.~~ **(Currently amended)** ~~The use according to claim 23 wherein said medicament is used for the~~ A method of treatment of human malignancies, comprising administering the pharmaceutical composition of claim 15.

~~25.26.~~ **(Currently amended)** ~~The use according to~~ method of claim 24-25, wherein said human malignancies are selected from the group consisting of: liver tumour-tumor, breast cancer, colon ~~or~~ cancer and rectal cancer.

~~26.27.~~ **(Currently amended)** A method of treatment of human malignancies comprising administering recombinant human arginase ~~into~~ to a patient.

~~27.28.~~ **(Currently Amended)** A method of treatment of human malignancies in a patient comprising administering a pharmaceutical composition that reduces the physiological arginine level in said patient to below 10 μ M for at least 3 days.